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## BRIEF REPORT

### OPEN

# Reduction in Serum Aquaporin-4 Antibody Titers During Development of a Tumor-Like Brain Lesion in a Patient With Neuromyelitis Optica: A Serum Antibody–Consuming Effect?

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### Abstract

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease of the CNS with severe involvement of the optic nerve and spinal cord. Highly specific serum IgG autoantibodies (NMO-IgG) that react with aquaporin-4 (AQP4), the most abundant CNS water channel protein, are found in patients with NMO. However, in vivo evidence combining the results of AQP4 antibody serum levels and brain pathology is lacking. We report a patient with NMO whose AQP4 antibody levels decreased simultaneously with clinical deterioration caused by the development of a tumor-like brain lesion. In the seminecrotic biopsied brain lesion, there was activated complement complex, whereas only very scattered immunoreactivity to AQP4 protein was detectable. The decrease in serum AQP4 antibody levels and the loss of AQP4 in the tumor-like lesion could represent a “serum antibody–consuming effect” during lesion formation.

**Key Words:** Aquaporin-4, Brain biopsy, Multiple sclerosis, Neuromyelitis optica, Serum level, Tumor-like lesion.

### CASE REPORT

A 64-year-old female patient fulfilled all diagnostic criteria for clinically definite neuromyelitis optica (NMO), including the

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Fahmy Aboulenein-Djamshidian and Romana Höftberger contributed equally to the study.

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The authors declare that they have no competing financial interests.

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presence of serum NMO-IgG autoantibodies (1, 2). She had a history of 3 episodes of longitudinally extensive ( $\geq 3$  vertebral segments) transverse myelitis in 2003, 2004, and 2005 (3–5). Her exacerbations were treated with high-dose intravenous methylprednisolone (ivMP) for 5 days. Cerebrospinal fluid analyses were performed twice in 2003 and 2004 and showed pleocytosis (22 and 44 cells/ $\mu$ L, respectively), with activated lymphocytes, monocytes, neutrophils, and eosinophils. Oligoclonal bands could not be detected in the cerebrospinal fluid (4–6).

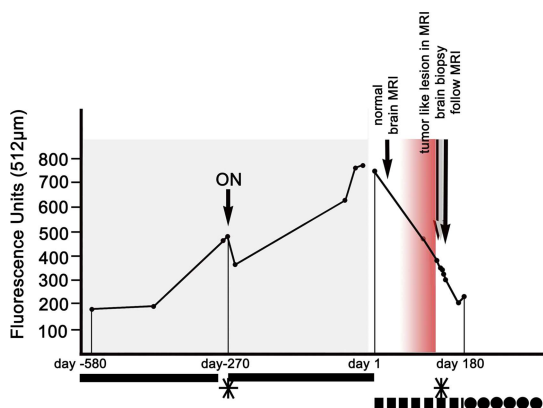
In 2005, the diagnosis was confirmed by detection of NMO-IgG through an indirect immunofluorescence (IF) assay at the Mayo Clinic Laboratories (Rochester, MN) (2). The test was obtained commercially. Aquaporin-4 (AQP4) antibody (AQP4-ab) titers were assessed with a fluorescence-based immunoprecipitation assay during follow-up, as described in detail (7). Long-term treatment with azathioprine and oral prednisolone was initiated (4, 5). Oral prednisolone was tapered and finally discontinued. Azathioprine dosing was adjusted to a lymphocyte count of 600 to 1,000 cells/ $\mu$ L.

In 2006, she had another exacerbation with optic neuritis (ON), and the same therapeutic regimen was applied (ivMP treatment followed by oral prednisolone, which was subsequently tapered and discontinued in May 2006). Serum AQP4 titers from 2005 to 2007 and the clinical course are shown in Figure 1 (left side of the graph, gray field); these have been included in previous reports (4–6).

Aquaporin-4 antibody titers increased continuously during an 11-month period and reached a maximum shortly before new clinical symptoms developed (December 2007 to March 2008). At this point, ongoing immunosuppressive therapy with azathioprine had to be switched to oral prednisolone because of elevated levels of liver enzymes (Fig. 1, Day 1). At the same time AQP4-ab titers began to decline. Brain magnetic resonance imaging (MRI) performed 3 weeks later showed normal results (6). Approximately 4 weeks later, the patient presented with agitation and prostration followed by progressing difficulties with visual perception and orientation (Fig. 1, red field). On clinical examination approximately 6 weeks later, the patient showed hemianopsia on the right; she was otherwise normal apart from mild spastic paraparesis (sequela of 3 previous attacks of myelitis). Neuropsychologic testing revealed major deficits in visuospatial and visuoceptive abilities. The clinical manifestations were caused by a large unique cystic seminecrotizing tumor-like brain lesion located bioccipitally and in the corpus callosum (Fig. 2).

### BRAIN BIOPSY

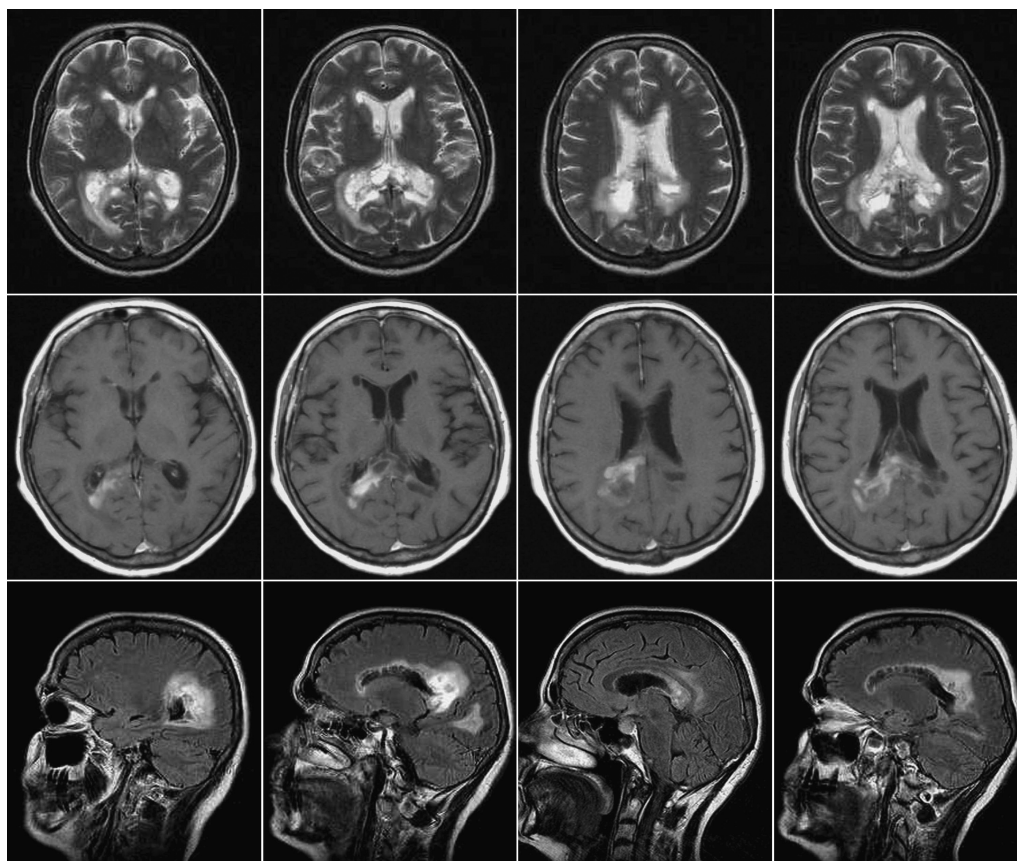
Three tissue cores from the stereotactic brain biopsy included material from the center and the edge of the tumor-like lesion. Two- to 4- $\mu$ m-thick adjacent serial sections of paraffin



**FIGURE 1.** Anti-AQP4-ab titers and clinical course. Black circles, AQP4-ab serum levels; black arrows, ON and brain MRI scans; gray arrow, brain biopsy; black rectangle, therapy with azathioprine; Day 1, day when azathioprine was discontinued because of elevated liver enzymes and therapy was switched to oral prednisolone; asterisks, 5-day course of ivMP 1 g/day (i.e. after ON and after brain biopsy); dashed line, therapy with low-dose prednisolone; dotted line, start of ongoing therapy with rituximab. The previous disease course and AQP4-ab titers were presented in detail elsewhere (3). The development of the large cystic NMO tumor-like lesion took several weeks to become evident on neuroimaging (red field).

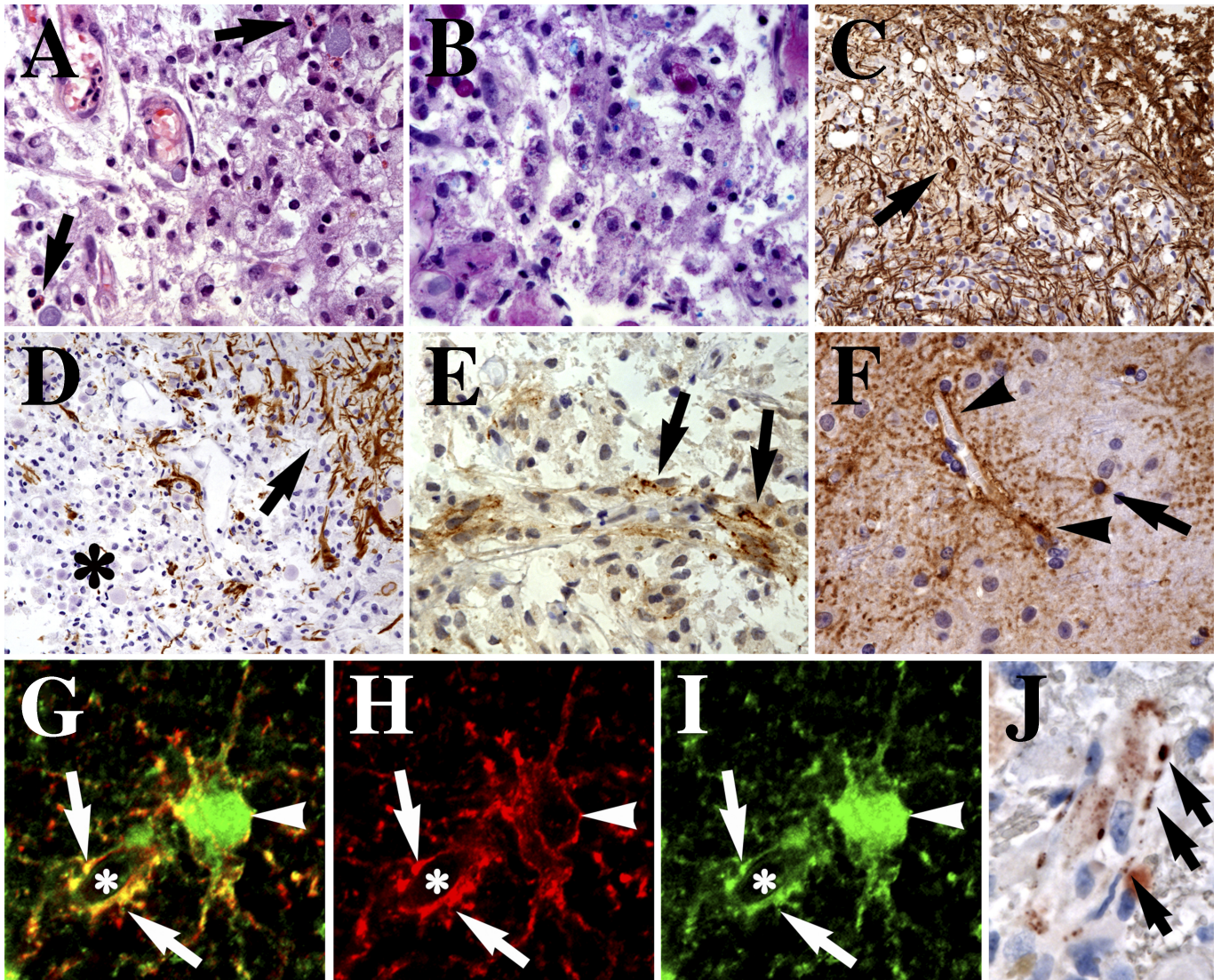
sections were stained with hematoxylin and eosin, Luxol fast blue, and Bielschowsky silver impregnation to assess inflammation, demyelination, and axonal pathology, respectively. Immunohistochemistry was carried out as previously described (8). The well-characterized antibodies included monoclonal (mouse) antibodies to CD3, CD4, CD8, CD20, CD79a, HLA-D, and phosphorylated neurofilament, and polyclonal (rabbit) antibodies to C9neo (activated complement complex [9]), glial fibrillary acidic protein, AQP4 water channel protein, and myelin basic protein. Immunohistochemistry and IF studies of the patient's serum taken 2 hours before the brain biopsy were performed on the biopsy material of the patient's tumor-like lesion and on normal brain, gastric, and kidney tissues. Established and modified protocols for immunohistochemistry and IF were performed on 2- to 4- $\mu$ m-thick paraffin sections, as previously described (2, 10). Fluorescein isothiocyanate-conjugated rabbit anti-human IgGAM was used as secondary antibody. For confocal laser microscopy, essentially the same laser scanning microscope and settings were used as previously described in detail (8, 10). Results of routine histology, immunohistochemistry, and IF studies are shown in Figure 3.

A first follow-up brain MRI was performed 11 days after the brain biopsy and 6 days after a course of ivMP (Fig. 1). Marked reductions of lesion size and gadolinium enhancement were found (data not shown). In a second follow-up



**FIGURE 2.** Tumor-like lesion in NMO. Axial and sagittal planes show the large extension of the tumor-like NMO lesion. First row, axial planes and T2-weighted MRI images; second row, axial planes and T1-weighted images with gadolinium; third row, sagittal planes and fluid-attenuated inversion recovery sequence.





**FIGURE 3.** Neuropathology of the tumor-like NMO lesion on brain biopsy and immunoreactivity of the patient's serum to AQP4. **(A)** Hematoxylin and eosin stain of the brain biopsy shows an inflammatory demyelinating lesion characterized by numerous macrophages, inflammatory infiltrates composed of neutrophilic and eosinophilic granulocytes (arrows), and lymphocytes. **(B)** Luxol fast blue-periodic acid Schiff stain shows active demyelination. **(C)** Immunohistochemistry for SMI31 shows that axons are relatively preserved within the lesion; arrow indicates an axonal spheroid. **(D)** Immunohistochemistry for glial fibrillary acidic protein reveals that there is widespread loss of glial fibrillary acidic protein in the lesion center (asterisk); astrocytes were well preserved (but activated) at the lesion border (arrow). **(E)** Immunohistochemistry with rabbit anti-AQP4-ab shows that AQP4 is markedly reduced in the NMO lesion. The very small AQP4 residual immunoreactivity is confined only to astrocytes and to the glia limitans perivascularis (arrows). **(F–I)** Normal parieto-occipital lobe tissue. Immunohistochemistry with rabbit anti-AQP4-ab shows intense staining for AQP4 in the cell bodies (arrow), processes, and foot processes of astrocytes that compose the glia limitans perivascularis (**F**, arrowheads). **(G–I)** Confocal images show labeling of rabbit anti-AQP4-ab (**H**, red) with the AQP4-ab-positive serum of the patient (**I**, green). Double labeling (**G**) shows colocalization at the glia limitans perivascularis (arrows) of small capillaries and within cell bodies of astrocytes (arrowhead) (yellow). Asterisk is within the capillary lumen. **(J)** Immunohistochemistry for activated complement complex (C3neo). Deposits of terminal complement complex are found around vessels (arrows) in the NMO lesion. Original magnification: **(A, B, E, F, J)** 400 $\times$ ; **(C, D)** 100 $\times$ ; **(G–I)** 630 $\times$ .

MRI 5 weeks after the biopsy, the lesion showed a further decrease in size (data not shown).

Our data support a crucial role for AQP4-ab/NMO-IgG autoantibodies in the pathogenesis of NMO (2, 7, 11). As in other patients with NMO, the tumor-like lesion in our patient developed at sites of high AQP4 expression (12). Aquaporin-4

antibodies have been shown to bind to AQP4 water channel protein on astrocyte foot processes, mainly at the blood-brain barrier. These antibodies are predominantly of the IgG<sub>1</sub> subtype and are capable of activating complement (13). Their targets may be destroyed by activation of the lytic complement complex or by opsonization (13). Within the center of the

lesion in our patient, there was nearly complete AQP4 loss; activated complement complex deposits and large numbers of activated macrophages were found, as has been observed previously on autopsy material by others (Fig. 3) (14–16). Eosinophils, CD4-positive lymphocytes, and plasma cells within the lesions also emphasize the antibody-driven nature of NMO. In a previous report, positive correlations between AQP4-ab titers and length of spinal cord lesions, severity of ON, or size of brain lesions were found (17). Our patient showed very high titers of AQP4-ab despite ongoing immunosuppressive therapy, whereas brain MRI performed before her recent relapse yielded normal results (Fig. 1) (4, 5). The increase in AQP4-ab titers before relapse corresponds to previous reports and occurred despite ongoing therapy with azathioprine (4, 17). The peak AQP4-ab titer seems to coincide with the initial development of the large tumor-like lesion (Figs. 1, 2) (17). The steady subsequent decline (of more than 50%) in AQP4-ab titers, even before the onset of high-dose steroid treatment (Fig. 1), is surprising and may indicate a “serum antibody-consuming effect” during lesion formation.

Consistent with previous findings, AQP4-ab titers continued to decrease after treatment with high-dose ivMP (4, 17). Reduced gadolinium enhancement indicated restoration of the blood-brain barrier 11 days after stereotactic brain biopsy and 6 days after a course of high-dose ivMP. It is possible that the low-dose prednisolone therapy contributed to the decline of AQP4 titers, but previous immunosuppressive therapy with azathioprine did not prevent the increase in AQP4 titers before tumor-like lesion formation (4). Our data are the first to combine serum and biopsy analyses, which lend further support to the potential role of AQP4-ab in the pathogenesis of NMO.

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